Long-term evaluation of motor function following intraneural injection of ropivacaine using walking track analysis in rats

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Background. There is a paucity of data regarding neurologic function following nerve injury. Our objective was the long-term evaluation of motor function following intraneural injection of ropivacaine in rats using the sciatic function index (SFI), derived from walking track analysis.

Methods. Rats were randomly assigned to one of four groups of 13 animals each. A needle was inserted under magnification into the left sciatic nerve and 0.2 ml of normal saline, formalin 15%, ropivacaine 0.2 or 0.75% were injected intraneurally. The right side was sham operated. Walking track analysis was performed the day before and on days 1, 4, 7, 11, 15, 18, 21, and 67 following intraneural injection. At the end of the experiment (day 67) a semi-quantitative evaluation of neuropathologic changes was performed by three independent observers.

Results. Animals treated with saline and ropivacaine (0.2 and 0.75%) had no detectable impairment of motor function at any time point. In contrast, rats treated with formalin had a complete loss of motor function immediately after the intraneural injection, which persisted until day 21 and returned to normal by day 67. Important histopathologic changes (score=2) with excellent inter-observer agreement were seen only in the group treated with formalin. This applied to both axonal degeneration and Schwann cell density evaluations.

Conclusions. These findings suggest that intraneural injections of ropivacaine at concentrations routinely used in clinical practice appear to have no deleterious effect on sciatic nerve motor function in this experimental rat model.

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The specialized structure of a peripheral nerve bundle is essential to normal sensory and motor function. Damage may be caused by mechanical, chemical, or ischaemic insults, which may occur either alone or in combination. In the ASA Closed Claims database in the 1980s and 1990s, temporary and permanent nerve damage was associated with 58 and 29% of peripheral nerve block claims, respectively.¹ Motor dysfunction has been reported after intraneural injection of local anaesthetics.² When it occurs, it is a debilitating condition with obvious clinical implications.

Ropivacaine, is a relatively new long acting amide local anaesthetic agent and is widely used in clinical practice at concentrations up to 0.75%. The potential peripheral neurotoxicity of inadvertent intraneural ropivacaine injection has never been studied. Peripheral nerves have a dual blood supply of intrinsic exchange vessels in the endoneurium and an extrinsic plexus of supply vessels in the epineurial space that cross the perineurium to anastomose with the intrinsic circulation.³ Ropivacaine, when topically applied to the rat sciatic nerve caused a greater reduction in neural blood flow compared with lidocaine and L-bupivacaine.⁴ Thus, local ischaemia may potentially result from intraneural injection of ropivacaine.

Histology and morphometric outcomes following nerve injury and/or repair correlate poorly with functional results.⁵ The Sciatic Functional Index (SFI) derived from walking track analysis in rats proved a reliable and easily quantifiable method of assessing sciatic nerve motor function.⁶
This study was undertaken to evaluate potential motor dysfunction resulting from intraneural injection of ropivacaine using the SFI in an experimental rat model.

Methods

The investigation was approved by the Ethics Committee for animal studies. Experiments were performed on 52 male Sprague–Dawley rats. Animals were housed in facilities thermostatically maintained at 20°C with artificial lighting for 12 h per day having free access to food and water. Animals were randomly assigned to receive 0.2 ml of either normal saline, formalin 15% (a fixative known to be toxic to all constituents of peripheral nerves), ropivacaine 0.2 or 0.75% intraneurally (n=13 per group).

Surgical procedure

All operations were carried out under ether and ketamine (100 mg kg⁻¹ intraperitoneally) anaesthesia. Both sciatic nerves were exposed by lateral incision on the thigh. Reflection of superficial fascia and muscle was performed in such a way as not to damage the underlying connective tissue layer, which contains the sciatic nerve. Following exposure of the sciatic nerve, a 30-gauge needle (Becton Dickinson micro-fine+, with a primary and secondary bevel of, respectively, 10 and 19°) was introduced parallel to the direction of nerve fibres in the centre of the nerve and 0.2 ml of the solution was injected intraneurally under direct vision using magnification. The needle was then withdrawn and the wound closed. Intraneural injection was performed only on the right sciatic nerve while the left was sham operated without being injected.

Assessment of motor function

Animals were tested in a confined walkway with a dark shelter at the end of the corridor by an observer unaware of group assignment. After three conditioning trials during which rats often stopped to explore the corridor they walked steadily to the dark shelter. The bottom of the track was lined with strips of paper, stained with bromophenol blue, which has been applied in absolute acetone and air dried before use. Tracks were obtained by wetting the rat’s hind feet with such a way as not to damage the underlying connective tissue layer, which contains the sciatic nerve. Following exposure of the sciatic nerve, a 30-gauge needle (Becton Dickinson micro-fine+, with a primary and secondary bevel of, respectively, 10 and 19°) was introduced parallel to the direction of nerve fibres in the centre of the nerve and 0.2 ml of the solution was injected intraneurally under direct vision using magnification. The needle was then withdrawn and the wound closed. Intraneural injection was performed only on the right sciatic nerve while the left was sham operated without being injected.

Walking track analysis was performed by custom software using a digitizing tablet and a personal computer. Calculations of the SFI and statistical analysis were performed using SAS program (SAS Inc., Cary, NC). Animals were randomly assigned to receive 0.2 ml of either normal saline, formalin 15% solution, ropivacaine 0.2 or 0.75% intraneurally.

Statistical analysis

All results are expressed as mean (SEM). Baseline values for SFI and weight as well as histological scores obtained
at the end of the experiment (D67) were compared between groups using one-way Kruskal–Wallis analysis of variance. Pairwise group comparisons were performed using Scheffe’s test. The evolution of the SFI and weight over time were compared between groups using repeated measures ANOVA. Inter-observer agreement was assessed using the weighted kappa coefficient. \( P < 0.05 \) was considered significant.

**Results**

Preoperative body weight (356 [46], 360 [48], 357 [46], and 357 [49] g for the groups treated with saline, formalin, ropivacaine 0.2 and 0.75%, respectively) as well as the change in this parameter throughout the study (data not shown) was similar among the groups.

Four animals belonging to the group treated with formalin showed evidence of auto-mutilation and were therefore excluded from the study for the entire period.

SFI profile in the four groups is displayed in Figure 2. Preoperatively, baseline values (D–1) were similar amongst the groups. Only animals treated with formalin had a complete loss of function at D1, D4, D7, D11, D15, D18, and D21 (\( P < 0.001 \) for each compared with baseline). They recovered by D67. A high density of Schwann cells was observed at this time compared with other groups (\( P = 0.0002 \)). In contrast, animals treated with saline, ropivacaine 0.2 and 0.75% had no detectable impairment of function, indicating that the two doses of ropivacaine tested (similar to saline) had no deleterious effect on sciatic nerve function.

Histological changes are summarized in Table 1. Important changes (score=2) were seen only in the group treated with formalin, whereas in all other group scores equaled 0 or 1. This finding applied to both axonal degeneration (group treated with formalin vs groups treated with ropivacaine 0.75% and saline, \( P = 0.0002 \)) and Schwann density evaluations (groups treated with formalin vs groups treated with saline, ropivacaine 0.2 and 0.75%, \( P = 0.0002 \)). In contrast, evaluation of endoneural fibrosis showed no difference between groups. Of note, inter-observer agreement was poor when assessing endoneural fibrosis across groups and also for all histological assessments within the groups treated with saline, ropivacaine 0.2 and 0.75%.

**Discussion**

The most important finding of our study was that intraneural injection of ropivacaine at concentrations routinely used in clinical practice had no deleterious effect on sciatic nerve motor function as measured by the SFI in this experimental rat model.
Various methods have been used in the past to assess recovery from peripheral nerve injury. These include morphometric, electrophysiological, biochemical, and histological analyses. Despite providing useful information, these tests do not measure the most important criteria, functional recovery. The reason why morphometric outcomes do not correlate with functional recovery may be a result of incomplete nerve regeneration or significant misdirection of the regenerating nerve fibres. 8,9 Few studies on peripheral nerve toxicity of local anaesthetics have provided information on nerve function. 10–14 The lack of precision of these evaluations led us to focus our attention on an accurate method for assessment of motor function of the rat sciatic nerve following injury caused by intraneural injection of ropivacaine.

The concept of walking track analysis as a method of assessing the function of the rat sciatic nerve has been described in detail elsewhere.6 Briefly, this gait analysis is based on the fact that rats normally walk on their digits and metatarsal footpads. Print length is therefore short in normal animals. Sciatic nerve lesions cause variable loss of both extensors and flexors of the foot. This deficit will cause the foot to drop to the ground and thus change the footprint. In this way footprints can be used to assess sciatic nerve function. Shortening of the footprint is thus a good sign of nerve recovery.

SFI has been widely used to assess motor nerve function and recovery following surgical repair, transection, crush injury, and intraneural injection.15 It has been shown to correlate with the severity of nerve injury in rats, with muscle strength; it has been validated as reliable, sensitive and reproducible.16–18

One strength of this study design is that it provides sequential information about dynamic motor function following nerve injury. As SFI compares the experimental and normal prints, each animal acted as its own control. Footprints remained normal on the sham operated side confirming the theory that SFI is not sensitive to non-nervous factors such as skin clips or muscle incision. The mechanism of nerve injury following intraneural injection is unclear. Mechanical trauma, toxic effects of the local anaesthetics, and ischaemia have been suggested. The lack of motor dysfunction following intraneural injection of saline observed in our study suggests that mechanical trauma (caused by the needle or by the injection itself) alone is not associated with functional deficit. This is in agreement with findings of other studies.11,12 Direct neurotoxicity of local anaesthetics is a function of exposure to excessive concentrations or doses.19 We demonstrated that ropivacaine at concentrations routinely seen in clinical practice are unlikely to produce motor deficit when accidentally injected intraneurally. Formalin, used as a positive control, led to motor deficit immediately after intraneural injection in all cases. Therefore, local ischaemia remains the most likely mechanism of nerve injury in clinical practice. Injecting a large enough volume of local anaesthetic into a relatively tight enclosed nerve sheath may increase intrafascicular pressure above capillary pressure.20 Similarly, a perineural haematoma may become increasingly compressive in certain areas. In addition, the blood supply of the brachial plexus is poor, which makes it even more vulnerable to injury.21 Beyond this, larger volumes injected under pressure may cause physical disruption of nerve structure.

There are, however, several limitations to this study. Sensory de-afferentation caused automutilation and thus rendered prints impossible to analyse in four rats injected with formalin. Furthermore, SFI assesses the sciatic nerve trunk and does not provide information on nerve branches.9 Depending on perineural rupture the consequences of nerve injury may vary. Intraneural injection is associated with severe injury, while extraneural injection results in minimal damage.22 At the injection site chosen in the present study, the rat sciatic nerve consists of two to three fascicules (one major and two minor) held together by loose connective tissue, the epineurium (Fig. 3). Thus, although intraneural injection was attempted, this could not be confirmed in all cases. However, major histological intraneural changes were clearly observed in seven out of nine rats belonging to the group treated with formalin. As the technique of injection was standardized it is likely that the proportion of intraneural injection was similar in all groups.

Regeneration is consistently present even in the most severely injured nerves. The rate of regeneration depends on the diameter of the axons, the proximity of the site of injection along the course of the nerve and the inherent toxicity. It is well known that rats have the ability to regenerate nerves following total degeneration therefore results obtained in this model should be extrapolated to humans with caution. However, in our study neural degeneration and subsequent regeneration was observed only in the group treated with formalin, which served as a control.
Although the human sciatic nerve is far larger than the rat sciatic nerve, it is interesting, that the minimal ratio of drug dose to body weight producing a full block of function seems to be the same for rats and humans. Volumes between 0.1 and 0.2 ml of lidocaine 1% injected perineurally have been repeatedly shown to reliably induce complete sensory and motor block in Sprague-Dawley rats weighing 300–400 g. In order to reproduce the clinical scenario of accidental intraneural injection, we injected the entire volume intraneurally.

SFI does not assess the sensory component following nerve injury. Because motor function is last to return (compared with sensory function), one can argue that dynamic assessment of motor function characterizes better recovery from nerve injury. For completeness, before we can conclude that there is no damage from accidental intraneural injections of ropivacaine during peripheral nerve blocks, further studies are warranted to determine possible sensory fibre dysfunction following intraneural injection, that is paw withdrawal to heat and mechanical stimuli. In addition, this thermal and mechanical testing may provide an index of changes in small fibre function.

There was significant inter-observer variation when comparing subtle histological changes within the groups treated with saline and ropivacaine (Table 1). This variability highlights the difficulty in using a semi-quantitative histological assessment as a means of quantifying nerve injury and makes these results difficult to interpret. Furthermore, the fact that nerve function in the above groups remained unaltered, underlines the limitation of this method in assessing nerve regeneration.

Animals treated with formalin demonstrated complete loss of function immediately following surgery. Recovery in this group occurred somewhere between the 21st and 67th day (Fig. 2). Histological changes were reliably reported as important when assessing axonal degeneration and Schwann cell density by the three observers (with a good degree of correlation in this group).

Using SFI, our study demonstrated that the intraneural injection of ropivacaine at concentrations routinely used in clinical practice has no deleterious effect on an experimental rat sciatic nerve motor function. Further studies to exclude potential transient or long lasting sensory deficit should follow before we can conclude that there is no damage from intraneural injections of ropivacaine.

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